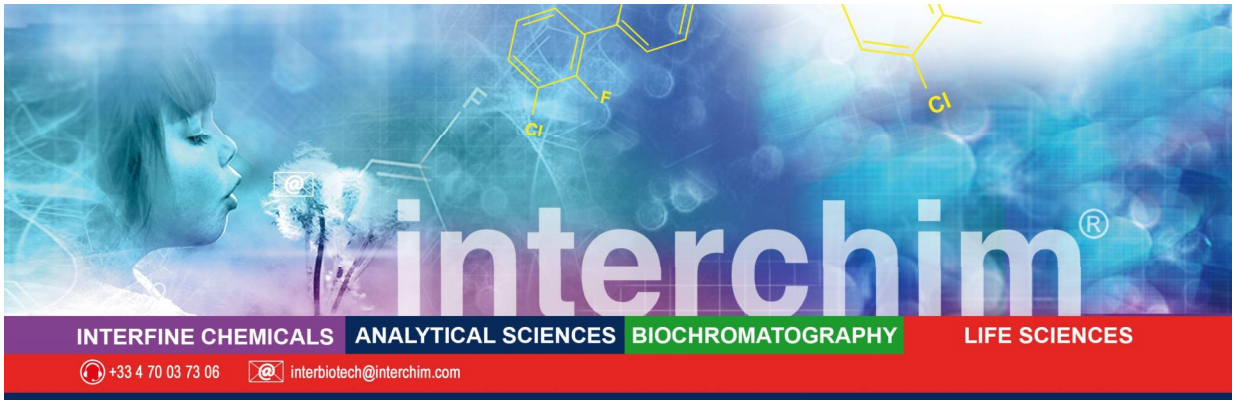

TMB Solutions (HRP Substrate for ELISA)

Products Description

Description	*Product Number*	*Applications*
TMB ELISA Peroxidase Substrate -Friendly Solution (stability 30 months)	UP664700, 200 ml UP664701, 500 ml UP664702, 1 L	ELISAs / HRP (classic applications)
TMB Solution « Check+ » (stability 30 months)	S08173, 200 ml S08174, 500 ml S08175, 1 L	ELISAs / HRP (with visual or optical control of deposits)
TMB Solution « Aqueous » (stability 24 months)	UPS08181, 200 ml UPS08182, 500 ml UPS08183, 1 L	ELISAs / HRP (notably diagnostic kits)

Storage : +4°C protected from light. Do not freeze. (K)

Uptima TMB solutions are chromogenic reagents for peroxidase, designed for manual or automated ELISA techniques. They contain 3,3',5,5'-tetramethylbenzidine (TMB), hydrogen peroxide (H_2O_2), and proprietary catalyzing and stabilizing agents. Reaction with peroxidase develops an intense blue color that can be read directly (at 650nm), or a deep yellow color (read at 450 nm) after stopping with an acid solution. Sensitivity is greater than classic substrates like OPD and ABTS, with very low background.

Our TMB substrates are available in 3 versions:
"**standard solution**" is the highly sensitive original version (the most sensitive tested reagent);
"**check+ solution**" indicator system provides assurance that substrate and acid stop have been added, improving overall quality control of assays performed manually or on automated immunoassay systems;
"**aqueous solution**" that does not contain organic solvents and is thus useful for diagnostic manufacturing (no shipping regulations).

Directions for Use

Protocol (please refer to specific protocol below for TMB check+ solution)

1. The ELISA assay may be performed according to your standard protocol. Wash the microplates thoroughly to remove any unbound peroxidase labeled probe (antibody, lectin, nucleotide…).

   Insufficient washing may lead to undesired background.
   Uptima recommends 4 washes in PBST (NaCl 150mM, phosphate 20mM, Tween20® 0.1%, pH7.5).

2. Add 150 µl of Uptima ready-to-use TMB substrate to each microplate well.

   Agitate slowly to homogenize.
   Incubate for 30 mins at room temperature, protected from light.
   See notes below for additional information (reagent storage [a], preparation [b], incubation optimization [c]).

3. **Immediate reading (blue color)**: useful for kinetic studies (not recommended with" Check+" solution)
   Read the optical absorbance at 630-650 nm. Recommended wavelength is 650 nm.

4. **Reading after reaction-stop (yellow color)**: sensitivity is increased 2 to 3 fold.
   Add 100 µl of stop solution (UPS29590) to each well. Positive wells become yellow.
   Read the optical absorbance at 450 nm. It is recommended to measure the absorbance immediately.
   It can be read up to 30min after stop-solution addition, but thereafter, signal may be decreased by 10%.
   For use of TMB check+ solution, also read at 540nm (the addition of the acid stop to the substrate produces a color change from colorless to pink). In peroxidase-negative wells (e.g., blank wells), the appearance of a pink color (measured at 540 nm) indicates that both substrate and acid stop have been added to the assay. The absence of a 540 nm signal indicates an invalid well, as either substrate or acid stop was not added to the well. In peroxidase-positive wells, the yellow color (absorbance at 450 nm) may obscure the visual color change of the indicator. However, the absorbance change at 540 nm can still be observed spectrophotometrically.

   See note [d] below for additional information

Additional Information

Uptima TMB substrates are optimized for direct and indirect ELISA techniques. They are not suitable for Immunohistochemistry or Western Blotting.
Uptima TMB solution standard was found the best amongst tested competitors, especially regarding sensitivity of detection in ELISA (ask for comparison of TMB NT-UP66478).
The reagent is very stable, at least 18 months under proper storage conditions.
Stringent manufacturing conditions ensure excellent lot-to-lot reproducibility.

Notes:

[a] Incorrect conditions of storage and operating may affect TMB performance:

- Exposure to light: TMB substrate is light sensitive. It should be stored in amber vials, and exposure of reagent to the light during the ELISA procedure should be limited (protect during incubation).
- Avoid important or frequent variations of temperature.
- The substrate is very sensitive to metallic ions. Only high quality plastic or glass should be used. Avoid the use of vials/caps with rubber seals: this could impair the results.

[b] TMB preparation

- The TMB solution is ready-to-use. For use, it is not necessary to reach room temperature.

Do not pipette the TMB directly from the bottle, and do not leave the bottle opened for long periods: fill a clean container first with the necessary solution volume to avoid contamination, and distribute to ELISA microplate (if not used immediately, protect from direct light exposure and keep at +4°C for no more than 1 day. It is preferable to only prepare the volume required).

[c] TMB incubation

- The volume per well and incubation duration may be adjusted depending on the detection system.
  If the staining is to rapid or intense, primary or secondary antibodies should be diluted, or staining time reduced.
  Do not dilute the TMB substrate.
- Stopping the reaction may be performed with various acid solutions. We recommend using our reagent UPS29590.
  H_2SO_4 1M allows noticeably higher signals, but the reading should be completed within 15minutes; otherwise there may be a 20-30% or more decrease of the signal and precipitate formation may be observed, affecting the accuracy and sensitivity of detection.

[d] 540nm reading for TMB Check+

- The absorbance at 540nm will vary with different substrate volumes and acid volumes. It may be necessary to conduct an experiment to determine the appropriate absorbance value at 540 nm indicative of substrate and acid addition with the assay's specific reagent volumes. A recommended experimental design is provided below:
  1. Pipette the desired volume of substrate into eight blank wells.
  2. Add the desired volume of acid stop to four of these wells.
  3. Read the absorbance of all wells at 540 nm.
  4. The average absorbance of wells receiving acid stop should be appropriate to indicate that substrate and acid stop have been added.

Related Documents and Products:

NT-66478c: TMB comparison of sensitivity , stability

See BioSciences Innovations catalogue and e-search tool:
*other Uptima reagents for ELISA procedures using TMB solutions:
Stop solution for TMB, UPS29590 (Specifications Sheet)
Peroxidase labeled Secondary Antibodies
Streptavidin labeled Peroxidase UP39588
PBS buffer, powder pack UP68723A and TBS buffer, powder pack UP74004A
PBS and TBS blends: with non fat milk GS4160 or with Tween20 GS4200
BSA UPQ84170 (powder) or UP900100(convenient solution 30%)
BioBlock Saturating agent (in TBS) N13650
SeaBlock Saturating agent UP40301 (no cross-reactivity with mammalian reagents)
RapidBlock saturating agent DZ7330 (protein-free, 5min blocking step)
FPlyte microplates

*Other substrates for HRP:
chromogenic: DAB tablets 732310
fluorigenic: ADHP (39423A, kit HS6241)
chemiluminogenic: UptiLight ECL Classic UP996190, High Sensitivity 36349A, UltraSensitive 996201

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